

The University of Chicago

THE RELATIONSHIP OF THE ADRENAL
AND THYROID GLANDS TO EXCISED
MUSCLE METABOLISM

A PART OF A DISSERTATION SUBMITTED
TO THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF MEDICINE

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The thyroid-adrenal relationship has been the subject of much investigation. Marine and Baumann reported that bilateral adrenalectomy of rabbits is usually followed by increased total respiratory metabolism (1), but that previous thyroidectomy lessened or prevented the rise in metabolism following adrenalectomy (2). Swingle et al. (3, 4, 5) reported that bilateral adrenalectomy of cats and dogs is followed by a fall in metabolism whether the thyroids are intact or totally removed. This study was undertaken to determine both the effect of bilateral adrenalectomy of mice on the metabolism of their excised muscle tissue and also the relationship of the thyroids to the observed effect, if any.

METHOD OF PROCEDURE. *Method of study.* In attacking the questions outlined in the introduction the respiratory metabolism of the excised skeletal muscle tissue of mice was determined under the following experimental conditions:

I. Bilateral adrenalectomy.

II. i. Bilateral thyro-parathyroidectomy.

ii. Adrenalectomy preceded by thyro-parathyroidectomy.

iii. Experimental hyperthyroidism induced by the administration of thyroxin.

iv. Adrenalectomy followed by experimental hyperthyroidism induced by the administration of thyroxin.

III. A series of experiments was also completed in which a comparison was made between the respiratory metabolism of i, the excised muscle tissue, and ii, the whole mouse as affected by adrenalectomy.

For every determination made on a mouse under experimental conditions one was made on a normal litter mate as control.

Animals used. The animals chosen for these series of experiments were mice. One of the reasons for this choice is because accessory cortical tissue is present in mice as in rabbits in varying amounts. Just as Marine and Baumann found in rabbits (1), so one finds in mice that bilateral adrenalectomy is fatal to some but not to others, the difference being due presumably

to the different amounts of accessory cortical tissue present. Those that live show varying degrees of adrenal insufficiency and are therefore suitable material for such a study as this.

Another reason for the choice of mice is the thinness of the muscle of their anterior abdominal wall. On account of this fact one is able to excise lateral strips of this muscle suitable for tissue metabolism experiments without having to resort to slicing, which undoubtedly wounds many cells and liberates tissue fluids either or both of which effects may exert an influence on the metabolism of the tissue. In making muscle slices great difficulty is encountered in obtaining pieces of uniform thickness, a difficulty which is avoided by the use of abdominal muscle. Not the least advantage gained is also the great saving of time in making the preparation.

Measurement of excised muscle metabolism. The aerobic metabolism of the excised abdominal muscle of the mice was determined by the Warburg differential method (6). By this method both the oxygen consumption, designated x_{O_2} , and the carbon dioxide production, designated x_{CO_2} , were determined and expressed in cubic millimeters of oxygen consumed, designated Q_{O_2} , and of carbon dioxide produced, designated Q_{CO_2} , per milligram of dried tissue per hour. The difference between Q_{CO_2} and Q_{O_2} gave the extra carbon dioxide produced and was a measure of the lactic acid produced by glycolysis, but, since the results obtained concern oxygen consumption only, the figures for lactic acid production have not been included.

The Warburg method has been modified as follows:

1. *pH of the medium.* The liquid medium used was a modification of Warburg's Ringer's solution (6, 7). The modification consisted in adding dextrose to make its concentration 0.2 per cent, and phenol red to make its concentration 0.00075 per cent, and bubbling through this dextrose-phenol red-Ringer's solution a gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide to bring it to a pH of 7.40. The concentration of phenol red in this solution was the optimum for colorimetric pH determinations by the Hastings and Sendroy method (8) of samples taken from the vessels at the conclusion of an experiment. The pH changes in the liquid media served as a check on the carbon dioxide production both from respiration and from glycolysis.

2. *Equilibrium.* The determinations of tissue metabolism were made with the vessels in a $37^\circ \pm 0.01^\circ$ water bath, which was kept in a $37^\circ \pm 0.2^\circ$ constant temperature room where the preparation of the vessels and tissue was also carried out. By simultaneously equilibrating the vessels containing the Ringer's solution with the 95 per cent oxygen and 5 per cent carbon dioxide gas mixture, the gas and liquid phases were brought close to their final equilibrium condition so that the time elapsing between the excision of the tissue and the attainment of equilibrium between tissue, liquid, and gas was a minimum. The time consumed in excising the tissue

and placing it in the vessels was usually 3 to 4 minutes, and the time allowed for it to attain equilibrium both in the air bath and in the water bath was usually 15 to 16 minutes, so that approximately 20 minutes elapsed after the mouse was sacrificed until measurement of its tissue metabolism began.

3. *Calculations.* Warburg (6) has deduced the following equations to express the measurement of tissue respiration by the differential method:

$$x_{O_2} = H \left(\frac{K_{CO_2} \times K_{O_2}}{K_{CO_2} + \gamma K_{O_2}} \right) \quad (i)$$

$$x_{CO_2} = \gamma x_{O_2} \quad (ii)$$

$$\gamma = \left(\frac{K_{CO_2} \times k_{CO_2}}{K_{O_2} \times k_{O_2}} \right) \times \left(\frac{HK_{O_2} - hk_{O_2}}{hk_{CO_2} - HK_{CO_2}} \right) \quad (iii)$$

where, x_{O_2} and x_{CO_2} represent the number of cubic millimeters, respectively, of oxygen consumed and carbon dioxide produced;

K_{O_2} and K_{CO_2} represent the oxygen and carbon dioxide constants, respectively, of the vessel containing the larger amount of liquid;

k_{O_2} and k_{CO_2} represent the oxygen and carbon dioxide constants, respectively, of the vessel containing the smaller amount of liquid;

h and H represent the heights in millimeters of the liquid in the manometers of the vessels containing, respectively, the smaller and larger amounts of liquid.

Calculations of x_{O_2} and x_{CO_2} by means of the foregoing equations are somewhat laborious and may be simplified by letting $K_{O_2} = a$, $K_{CO_2} = b$, $k_{O_2} = c$, $k_{CO_2} = d$. Then, if the value of γ given by equation iii is substituted in equations i and ii, and if the letters a , b , c , d , are substituted for the constants in the resulting equations, it may be shown that

$$x_{O_2} = \left[\frac{acd}{ad - bc} \right] h - \left[\frac{abc}{ad - bc} \right] H \quad (iv)$$

$$x_{CO_2} = - \left[\frac{bcd}{ad - bc} \right] h + \left[\frac{abd}{ad - bc} \right] H \quad (v)$$

When the numerical values of the constants are substituted in place of the letters a , b , c , d , in equations iv and v, the resulting equations are simple binomial expressions involving only h and H with their numerical coefficients. For example, for one pair of vessels used,

$$x_{O_2} = - 2.32 h + 1.95 H \quad (vi)$$

$$x_{CO_2} = + 3.96 h - 2.21 H \quad (vii)$$

4. *Sensitivity.* In the x_{O_2} and x_{CO_2} expressions of the preceding section, it may be shown that the numerical coefficients of h and H in expressions vi

and vii will vary with a , b , c , and d of expressions iv and v, and that the latter will vary with the gas and liquid content of the vessels. A vessel pair, M and N , was selected, whose volumes to the manometer menisci were 12.17 and 25.64 cc., respectively, and compared with a vessel pair, A and B , whose volumes were 16.33 and 16.24 cc., respectively. When vessel pair A and B , contained 3 and 9 cc. of Ringer's solution, respectively,

$$x_{O_2} = - 2.32 h + 1.95 H$$

and

$$x_{CO_2} = + 3.96 h - 2.21 H.$$

When vessel pair, M and N , contained 3 and 8 cc. of Ringer's solution, respectively,

$$x_{O_2} = - 13.3 h + 27.2 H$$

and

$$x_{CO_2} = + 16.8 h - 32.3 H.$$

When comparable pieces of abdominal muscle of mouse were placed in each of the 4 vessels, there was great discrepancy in the Q_{O_2} and Q_{CO_2} values obtained by the two vessel pairs. When, however, M and N contained 3 and 16 cc. of Ringer's solution, respectively, so that

$$x_{O_2} = - 1.30 h + 2.30 H$$

and

$$x_{CO_2} = + 2.52 h - 2.79 H,$$

there was good agreement in the Q_{O_2} and Q_{CO_2} values obtained by the two vessel pairs. In the first case, where there was no agreement the coefficients of h and H for the M and N vessel pair were large; in the second case where there was good agreement the coefficients of h and H for the M and N vessel pair were small; in both cases the coefficients of h and H for the A and B vessel pair were small. Since the manometer readings will be maximum for minimum values of the coefficients, it appears desirable for tissues respiring at the rate of abdominal muscle of mice that the quantity of gas and liquid in the vessel pair be such as to give as small coefficients of h and H as are practicable. Vessel pair A and B , which fulfilled these conditions, was one of several similar pairs used in the following experiments.

EFFECT OF ADRENALECTOMY ON THE METABOLISM OF EXCISED ABDOMINAL MUSCLE OF MICE. *Operative procedure.* Bilateral adrenalectomies were performed on the mice under light ether anesthesia by the dorsal route. Reflecting the skin bilaterally from a central dorsal incision, bilateral incisions were made through the lateral abdominal walls just dorsal to the last

ribs. With this exposure it was possible after a little practice to find and excise the adrenals in a minute or two without causing other trauma or hemorrhage than that due to the required incisions and excisions. The mouse usually carried on for 3 or 4 days after the operation much as if nothing had happened, and the symptoms that subsequently appeared were those characteristic of adrenal insufficiency, not of operative procedure.

Symptoms of adrenal insufficiency. The usual effects of bilateral adrenalectomy observed in other animals, especially rats and rabbits, were also observed in mice. There were no symptoms observable on ordinary inspection before 4 or 5 days. Then about 20 per cent of the mice died within 3 to 10 days showing the following symptoms: they began to eat less well and finally wholly refused food; their weight markedly decreased and their rectal temperature fell from 2 to 3 degrees below normal; they indulged in almost no voluntary activity, and in some cases the weakness manifested itself in inability to use the hind quarters; a few died in convulsions. The behavior of the other 80 per cent appeared normal, but when compared with their normal litter mates they showed differences in weight gains, voluntary activity, endurance tests, and muscle tissue metabolism.

Weight gains. Mice that survived bilateral adrenalectomy for longer periods than 7 to 10 days usually showed an initial weight loss followed by a weight gain until about 40 days after operation. During the 40 to 70 day period after adrenalectomy most of these mice either made no gain or suffered a loss in weight, whereas their unoperated litter mates continued to gain weight. This is in accord with Jaffe's observation (9) that one of the symptoms of adrenal insufficiency in rats is progressive emaciation with marked or complete absence of fat.

Voluntary activity. Several mice that survived bilateral adrenalectomy until sacrificed had their voluntary activity measured by the number of revolutions they made per day in a revolving wheel. It was found that the average number of revolutions per day during the 40 to 70 day period after adrenalectomy was much less than before or up to the fortieth day after.

Endurance tests. Several mice that survived bilateral adrenalectomy from 30 to 70 days were subjected to endurance tests. Both the operated mouse and its litter mate control were kept in place on the outside of a revolving wheel, and the wheel was revolved slowly requiring the mice to keep in motion to maintain their positions. In no case was the operated mouse able to make as many revolutions as its normal litter mate. In most cases the operated mouse soon showed signs of exhaustion and generally collapsed after a few hundred revolutions and later succumbed, whereas the normal mouse would soon voluntarily resume its activity on being placed inside the wheel. This is in accord with the observation of Gans and Miley (10) who showed that the total work done by adrenalectomized rats before complete fatigue set in was only one-sixteenth of that carried out by the controls.

Tissue metabolism. The effect of bilateral adrenalectomy on the metabolism of the excised abdominal muscle of the surviving mice was determined at various intervals from 11 to 111 days after adrenalectomy. The differences in metabolism of 43 adrenalectomized mice as compared with that of their litter mate controls are shown in figure 1. It will be noted that beginning about the fortieth day and continuing until about the seventieth day after adrenalectomy the differences were large and positive, whereas before this period the differences were small and negative, and after this period small and either positive or negative. During the 40 to 70 day period the average of 18 oxygen consumption values of the adrenalectomized mice was 6.23, which was significantly greater than the value of 4.62 obtained for their litter mate controls. Whether the increased value was due, as Marine and Baumann (1) thought, to uninhibited activity of the thyroids will be left for fuller consideration in the next section.

THE EFFECT OF EXPERIMENTAL HYPO- AND HYPERTHYROIDISM ON THE METABOLISM OF EXCISED ABDOMINAL MUSCLE OF ADRENALECTOMIZED MICE. In order to test the hypothesis that the increased metabolism of excised abdominal muscle of mice occurring after bilateral adrenalectomy is due to increased activity of the thyroid glands, three series of experiments were performed in order to study first, the effect of bilateral thyroidectomy alone; second, the effect of adrenalectomy preceded by thyroidectomy; third, the effect of experimental hyperthyroidism both with and without previous adrenalectomy.

Effect of bilateral thyro-parathyroidectomy. Since thyroidectomy of mice also involved parathyroidectomy, an attempt was made to prevent tetany by following the treatment used by Rockwell (12) on rats. This consisted essentially of feeding the mice on a predominantly buttermilk diet both before and after operation. Under this system of feeding no tetany was ever observed. Some fatalities occurred, but they were during or soon after the operation and seemed to be due mainly to hemorrhage. When the surviving mice were finally sacrificed for their abdominal muscle, autopsies showed that complete removal of the thyroids was rarely accomplished.

Symptoms of thyroid insufficiency other than metabolic were few. It is perhaps worth mentioning, however, that loss of hair frequently occurred and in some cases there was an excessive gain in weight.

Tissue metabolism experiments were performed on 12 of these mice and their litter mate controls. The average oxygen consumption value of the thyroidectomized group was 4.27, which was significantly less than the value, 6.02, obtained for the control group.

Effect of adrenalectomy preceded by thyro-parathyroidectomy. Bilateral thyro-parathyroidectomies were performed as described in the preceding section. About 3 weeks after this operation bilateral adrenalectomies were also performed. Though the majority of the mice survived the first opera-

tion, few, if any, seemed able to survive the second operation without treatment with thyroxin. The procedure was therefore modified as follows: after thyroidectomy subcutaneous injections of 0.1 mgm. of thyroxin¹ in 0.1 cc. solution were made three times a week. By this procedure 13 mice were kept alive from 50 to 70 days after adrenalectomy.

The tissue metabolism of these 13 mice and their litter mate controls was determined at various intervals during the 50 to 70 day period after adrenalectomy. The average oxygen consumption value of the thyro-parathyroid-adrenalectomized, thyroxin-treated group was 5.69, which did not differ significantly from the value, 5.90, obtained for the control group. If it can be regarded that the dose of thyroxin administered in

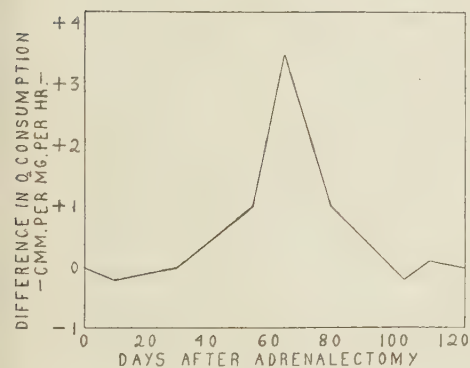


Fig. 1

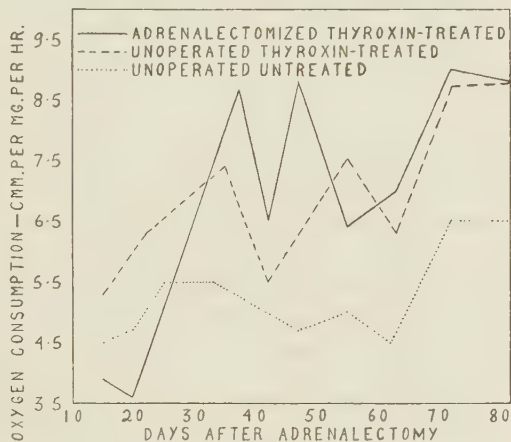


Fig. 2

Fig. 1. The difference between the oxygen consumption of normal and adrenalectomized mice.

Fig. 2. A comparison of the effect of thyroxin on the oxygen consumption of normal and adrenalectomized mice.

these experiments was just adequate to replace the loss of thyroid secretion due to thyroidectomy, and since this amount was kept constant throughout the course of the experiments, the absence of any difference in tissue metabolism following adrenalectomy adds weight to the hypothesis that the increase in metabolism observed in the first series was due to increased thyroid activity and not simply to absence of the adrenal glands.

Effect of experimental hyperthyroidism. Hyperthyroidism was produced experimentally in the mice by subcutaneous injection of 0.1 cc. solution of 0.1 mgm. of thyroxin daily for various periods. Both an adrenalectomized mouse and its litter mate were so injected and their aerobic metabolism

¹ Natural thyroxin from British Drug House, London.

compared with that of a litter mate control. Twelve such experiments resulted in an average oxygen consumption of 6.75 for the adrenalectomized, thyroxin-treated group and of 6.74 for the unoperated, thyroxin-treated group, both of which values were significantly greater than the value 5.01 obtained for the control group. Though the values 6.75 and 6.74 (for the two experimental groups) were approximately equal, it may be seen from figure 2 that the oxygen consumption of the adrenalectomized, thyroxin-treated group was less than that of the unoperated, thyroxin-treated group during the first 26 days, but greater during 38 to 74 days after adrenalectomy. Apparently, the adrenalectomized mice were more susceptible to thyroxin administration during the latter period. This result as well as the following symptoms of hyperthyroidism appear to be in accord with the Marine-Baumann hypothesis that the increased metabolism was due to increased activity of the thyroids.

More marked symptoms of hyperthyroidism were developed by 3 of the original group of 15 adrenalectomized, thyroxin-treated mice, namely, diarrhea, increased temperature, and extreme loss of weight. These 3 eventually succumbed, whereas their unoperated litter mates under similar dosage did not exhibit such marked symptoms and did not succumb.

Another symptom of hyperthyroidism was shown by the unoperated, thyroxin-treated mice in lessened voluntary activity when placed in a revolving wheel. The same tendency was also exhibited by mice that showed increased metabolism following adrenalectomy.

It will be observed that the 38 to 74 day period after adrenalectomy during which the tissue metabolism of the operated, thyroxin-treated group exceeded that of the unoperated, thyroxin-treated group was approximately the same as the 40 to 70 day period during which an increased tissue metabolism following adrenalectomy was observed, and as the 40 to 70 day period during which a decrease was shown in voluntary activity.

A COMPARISON OF THE OXYGEN CONSUMPTION OF INTACT MICE AND THEIR EXCISED ABDOMINAL MUSCLE FOLLOWING ADRENALECTOMY. *Oxygen consumption of the intact mouse and its excised muscle.* The oxygen consumption of the intact experimental mice was measured by the Davis-van Dyke method (12) before adrenalectomy and also at periods of 43 to 65 days after adrenalectomy. At the latter time oxygen consumption measurements were also made on their intact litter mate controls. Immediately after this final measurement of the total metabolism of both mice their excised abdominal muscle metabolism was also measured. Thirteen such experiments were carried out. The average oxygen consumption of both the control and the adrenalectomized mice before operation was approximately 37.5 liters per kilo of body weight per 24 hours. The average oxygen consumption of the adrenalectomized group after adrenalectomy was 44 liters per kilo of body weight per 24 hours, an increase of 16.9 per cent over the

value obtained for the control group. The oxygen consumption of the excised muscle of the control group averaged 4.37, of the adrenalectomized group 5.12, the increase being 17.8 per cent. Thus, both the total and tissue metabolism increased during the 43 to 65 day period after adrenalectomy, and the increases were of approximately the same order of magnitude.

Effect of adrenalectomy on total respiratory metabolism. Since total respiratory metabolism and excised muscle metabolism appear to be related phenomena, the effects of adrenalectomy on total respiratory metabolism deserve consideration.

Besides Marine and Baumann, to whom reference was made in the introduction as having obtained an increase in the metabolism of rabbits following adrenalectomy, Golyakhovski (13) reported an increase in the heat production of dogs after most of the blood supply to the adrenals had been blocked by mass ligation, and Scott (14) reported a slight increase in the metabolism of some cats and no change in others after sub-total injury of the adrenals.

Besides Swingle et al., to whom reference was made in the introduction as having obtained a decrease in the metabolism of cats and dogs following bilateral adrenalectomy, Gradinescu (15) had previously made a similar report, and Scott (14), and Aub, Forman and Bright (16) also reported a fall in metabolism of cats following total removal of the adrenals.

The effects of adrenalectomy on total respiratory metabolism seem somewhat discrepant according to the observations of the workers quoted in the preceding paragraphs. If, however, the animals studied are divided into two groups, the one including dogs and cats and the other mice and rabbits, most of the discrepancies disappear, for the two groups differ markedly in the fact that most mice and rabbits have enough accessory cortical tissue to prevent death after bilateral adrenalectomy, but not enough to prevent some of the effects of adrenal insufficiency, whereas cats and dogs have so little accessory cortical tissue that their adrenal insufficiency is so acute after bilateral adrenalectomy that death invariably results within 2 to 10 days. The reports agree that in dogs and cats bilateral adrenalectomy is followed by decreased metabolism, that in mice and rabbits bilateral adrenalectomy is followed in most cases by increased metabolism, the result, whether increase, decrease, or neither, depending upon the amount of active cortical tissue present. The dogs and cats which from various causes suffered sub-total injury of the adrenals really belong in the mice and rabbit group and therefore variable results might be expected, depending on whether the extent of the injury rendered their adrenal insufficiency lethal or sub-lethal. From these considerations it appears that sub-lethal insufficiency is likely to result in an increased metabolism which does not occur in the absence of the thyroids, and that lethal insufficiency always results in decreased metabolism whether the thyroids are present or not.

CONCLUSIONS

Summary of results. The results of the 3 series of experiments described in the preceding sections have been summarized in table 1 and may be stated as follows:

1. The experiments of series I showed that during the 40 to 70 day period after adrenalectomy of mice the aerobic metabolism of their excised abdominal muscle is increased. Before and after that period there is no such increase.

TABLE 1

A comparison of the oxygen consumption of 7 groups of normal and experimental mice

GROUP NUMBER	NUMBER OF EXPERIMENTS IN GROUP	EXPERIMENTAL TREATMENT	DAYS AFTER TREATMENT	MEAN O ₂ CONSUMPTION OF EXCISED MUSCLE OF GROUP		DIFFERENCE OF MEANS	PROBABLE ERROR OF DIFFERENCE OF MEANS
				Normal	Experimental		
				cu. mm. per mgm. per hr.	cu. mm. per mgm. per hr.	cu. mm. per mgm. per hr.	cu. mm. per mgm. per hr.
I	18	Adrenalectomy	40-70	4.62	6.23	1.61	0.17
	i 12	Thyroidectomy	16-92	6.02	4.07	1.75	0.20
	ii 13	Thyroidectomy preceding adrenalectomy	50-70	5.90	5.69	0.21	0.17
II	iii 12	Thyroxin administration	5-39	5.01	6.74	1.73	0.20
	iv 12	Adrenalectomy preceding thyroxin administration	15-90	5.01	6.75	1.74	0.40
							PER CENT INCREASE
III	i 13	Adrenalectomy	43-65	4.37	5.12	0.75	17.8
	ii 13	Adrenalectomy	43-65	37.5*	44.0*	6.5	16.9

* Liters of O₂ consumed per kilo of intact mouse per 24 hours.

2. The experiments of series II, i and ii, showed that if adrenalectomy is preceded by thyroidectomy there is no increase in the aerobic metabolism of abdominal muscle of mice during the 40 to 70 day period after adrenalectomy.

3. The experiments of series II, iii and iv, showed that during the 40 to 70 day period after adrenalectomy, adrenalectomized mice show an increased susceptibility to thyroxin administration.

It was also shown that during the 40 to 70 day period after adrenalectomy the voluntary activity of adrenalectomized mice is similar to that of unoperated, thyroxin-treated mice.

4. The experiments of series III showed that during the 40 to 70 day period after adrenalectomy the total respiratory metabolism and the excised abdominal muscle metabolism show corresponding increases.

It may be noted in table 1 that the average oxygen consumption values of the excised abdominal muscle of the 5 groups of normal mice have varied as follows: 4.62, 6.02, 5.90, 5.01, 4.37. The latter value was obtained in the early spring for mice 6 to 7 months old, while the largest value was obtained in the fall for mice about 3 months old. Perhaps the variation can be accounted for partly by differences in age, season, strain from which the mice derived, and the time consumed preparing the tissue for the experiment if it varied much from the 20 minutes usually taken. While the conditions have, no doubt, varied from experiment to experiment, it is important that they were the same for both control and experimental mouse in any one experiment and that the differences between the two groups were consistent throughout all the experiments.

Interpretation of results. The results of this study suggest a relationship between the adrenal and thyroid glands which Marine and Baumann (1) at first explained as follows: they supposed that the adrenals exerted some inhibitory influence on the thyroids and that, when this inhibition was removed, the activity of the thyroids was increased. More recently, Marine (17) suggested that the increased production of thyroid hormone in Graves' disease was a physiological attempt toward compensation of deficiency of some function of the adrenal cortex and sex glands. It seems quite probable that the increased metabolism of mice following adrenalectomy is also a physiological attempt toward compensation of adrenal insufficiency by an increased production of thyroid hormone, and that this compensation continues until accessory adrenal tissue develops sufficient activity to make further compensation unnecessary. Why this increased activity of mouse thyroid is not noticed until 40 days have elapsed may be due to the fact that mice are very resistant to the metabolic effect of thyroxin, requiring as much as a rabbit 75 times as heavy in order to produce a corresponding effect.

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